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(54) **METHOD FOR PREVENTING AND TREATING *SALMONELLA* TYPHIMURIUM INFECTION**

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None
See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to a composition comprising STP-1, a bacteriophage isolated from nature, capable of infecting *Salmonella Typhimurium* so as to kill the same as an active ingredient, and a method for preventing and treating *Salmonella Typhimurium* infection using the said composition. According to the present invention, the bacteriophage STP-1, an active ingredient of the composition, has a killing activity against *Salmonella Typhimurium* and has the genome represented by SEQ. ID. NO: 1.

2 Claims, 2 Drawing Sheets

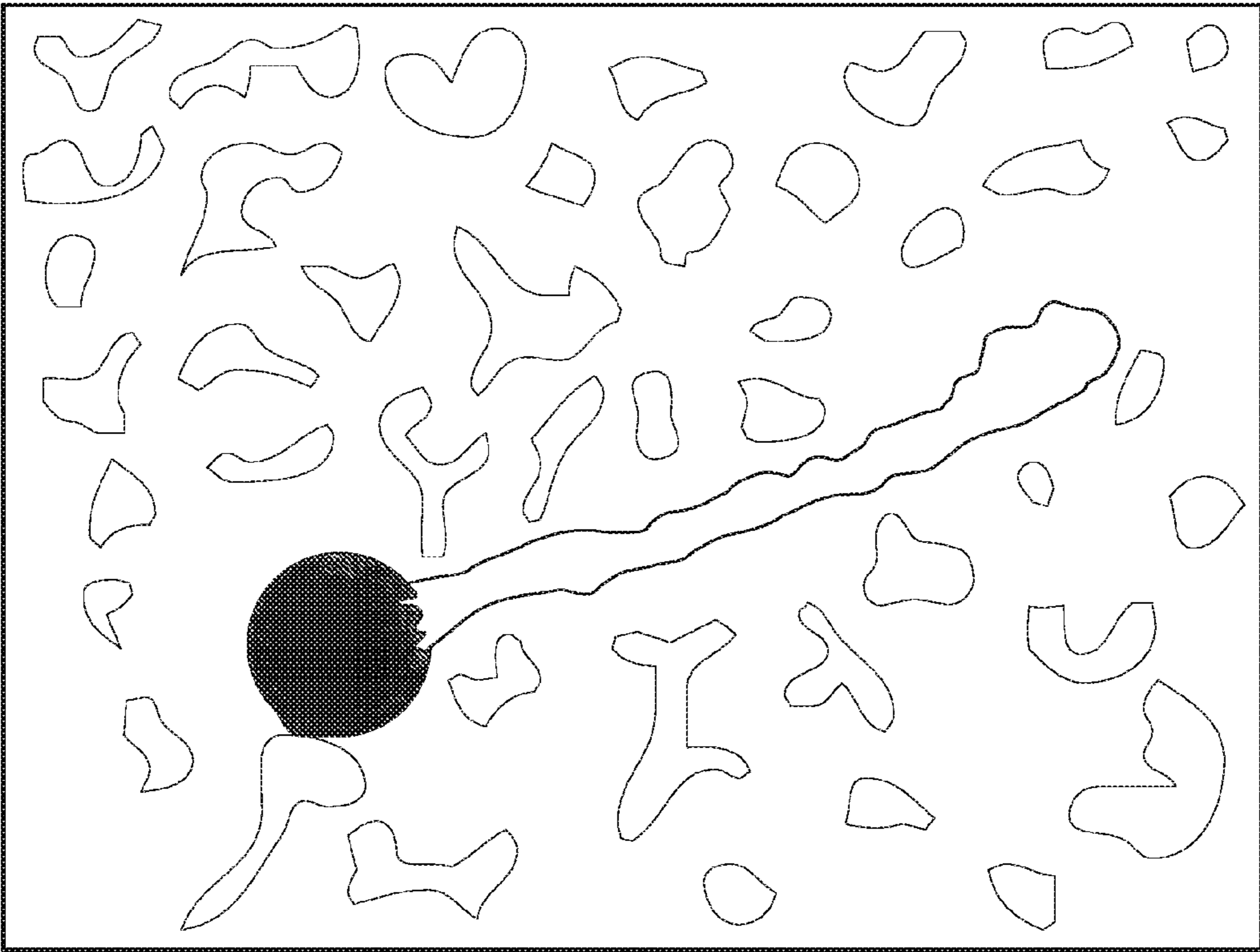


FIG. 1

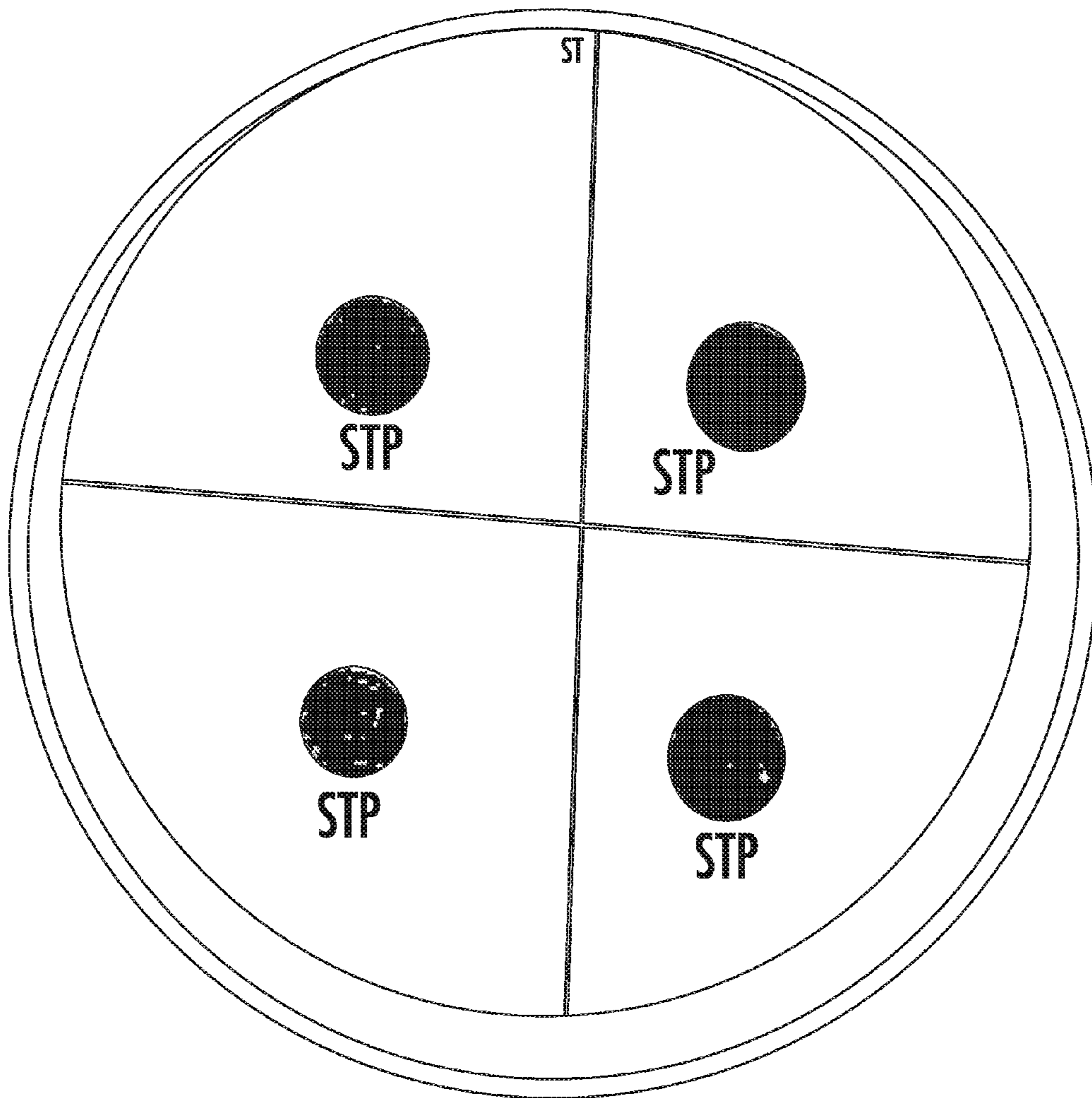


FIG. 2

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**METHOD FOR PREVENTING AND
TREATING *SALMONELLA* TYPHIMURIUM
INFECTION**

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a composition usable for preventing or treating *Salmonella Typhimurium* infection comprising a bacteriophage, isolated from nature and capable of infecting *Salmonella Typhimurium* so as to kill the same as an active ingredient, and a method for preventing and treating *Salmonella Typhimurium* infection by using the said composition. More precisely, the present invention relates to a bacteriophage isolated from nature which is characterized by having the genome represented by SEQ. ID. NO: 1 and is capable of killing *Salmonella Typhimurium* specifically, a composition usable for preventing and treating *Salmonella Typhimurium* infection comprising the said bacteriophage as an active ingredient, and a method for preventing and treating *Salmonella Typhimurium* infection by using the said composition.

2. Description of the Related Art

Salmonella is similar to *E. coli* in the aspects of morphology or physiology but is categorized in an independent genus for the convenience in clinical use by the proposal of K. Kauffmann et al. *Salmonella* has been isolated from enteritis and gastroenteritis patients and from animals with diverse diseases since *Salmonella Choleraesuis* was first isolated from a pig died of hog cholera by Salmon and Smith in 1885. *Salmonella* has also been isolated from healthy animals such as chicken, cow, pig, goat, dog, and cat and from our environment.

More than 2,000 serotypes of *Salmonella* have been reported so far and it can be largely divided into two groups, one of which is the group that has host specificity and the other of which is the group that does not have host specificity. *Salmonella* is Gram-negative bacilli and spore is not formed. It is a parasite living in a variety of animals.

Salmonella infection is generally called salmonellosis, which carries the symptoms of rough skin, anorexia, conjunctivitis, depression, loose feces, splenomegaly, and even death.

The most frequently found *Salmonella* in pig industry is *Salmonella Typhimurium*. The loss and damage caused by *Salmonella Typhimurium* infection in livestock industry, particularly in pig industry, is huge. Therefore, it is urgently requested to develop a novel method to prevent and treat *Salmonella* infection efficiently.

The utilization of bacteriophage is now highly drawing our attention as an effective way of treating bacterial disease. In particular, our interests in bacteriophage grow with the preference of nature-friendly method. Bacteriophage is an extremely small microorganism infecting bacteria, which is generally called phage in short. Bacteriophage replicates within bacteria after infection. Upon completion of the multiplication, offspring bacteriophages are coming out of the host cells with destroying the host bacteria. The infection of bacteriophage in bacteria is very unique and specific, so only specific bacteria can be infected with a specific bacteriophage. That is, there is a limitation in bacteria that can be infected with bacteriophage. Thus, bacteriophage can only kill specific target bacteria without affecting any other bacteria.

Bacteriophage was first found in 1915 when English bacteriologist Twort was studying on the phenomenon that *micrococcus* colony was being melted clearly by some reasons. And also, French bacteriologist d'Herelle noticed that

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Shigella dysenteriae was melted by something in filtrate of dysentery patient's feces and afterwards he discovered bacteriophage independently by the following study and named it bacteriophage which meant 'eating bacteria'. Since then, bacteriophages corresponding to different pathogenic bacteria including *Shigella*, *Salmonella* and *Vibrio cholerae* have been continuously reported.

Owing to its capability of killing bacteria, bacteriophage has been in the center of our interest to fight with bacterial infection and studies followed thereon. However, since Fleming found out penicillin, antibiotics have been supplied and the study on bacteriophage has been limited in some east European countries and old Soviet Union. It was not until 2000 that the conventional antibiotics demonstrated their problems in use because of increasing antibiotic-resistant bacteria. So, once again, bacteriophage draws out attention as an alternative anti-bacterial agent that can take the place of the conventional antibiotics.

The present inventors have tried to develop a composition usable for preventing or treating *Salmonella Typhimurium* infection by using a bacteriophage isolated from nature and capable of killing *Salmonella Typhimurium* selectively and tried further to establish a method to prevent or treat *Salmonella Typhimurium* infection by using the said composition. As a result, the present inventors succeeded in isolation of a proper bacteriophage from nature and obtainment of a sequence of the genome distinguishing the phage from others, leading to the completion of the present invention by confirming that the composition developed by the inventors could be effectively used for the prevention and treatment of *Salmonella Typhimurium* infection.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a novel bacteriophage capable of killing *Salmonella Typhimurium* selectively.

It is another object of the present invention to provide a composition usable for prevention of *Salmonella Typhimurium* infection comprising the said bacteriophage as an active ingredient which is capable of killing *Salmonella Typhimurium* selectively by infecting *Salmonella Typhimurium* and to provide a method for prevention of *Salmonella Typhimurium* infection using the same.

It is also an object of the present invention to provide a composition usable for treatment of *Salmonella Typhimurium* infection comprising the said bacteriophage as an active ingredient which is capable of killing *Salmonella Typhimurium* selectively by infecting *Salmonella Typhimurium* and to provide a method for treatment of *Salmonella Typhimurium* infection using the same.

It is further an object of the present invention to provide a disinfectant for the prevention and treatment of *Salmonella Typhimurium* infection using the said composition.

It is also an object of the present invention to provide a water additive for the prevention and treatment of *Salmonella Typhimurium* infection using the said composition.

It is also an object of the present invention to provide a feed additive for the prevention and treatment of *Salmonella Typhimurium* infection using the said composition.

To achieve the above objects, the present invention provides a composition comprising a bacteriophage as an active ingredient which is capable of destroying *Salmonella Typhimurium* by infecting *Salmonella Typhimurium*, and a method for preventing and treating *Salmonella Typhimurium* infection by using the said composition.

The present invention also provides a disinfectant, a water additive, and a feed additive that can be used for the prevention or treatment of *Salmonella Typhimurium* infection.

Advantageous Effect

As explained hereinbefore, the composition of the present invention and the method for preventing and treating *Salmonella Typhimurium* infection using the same have an advantage of high specificity against *Salmonella Typhimurium*, compared with other conventional chemical compositions and methods using thereof. That is, this composition does not have any effect on other useful resident flora and can be used only for the purpose of prevention and treatment of *Salmonella Typhimurium* infection. Thus, side effects are hardly accompanied. In general, when other chemicals such as the conventional antibiotics are used, general resident bacteria are also damaged, resulting in the decrease of immunity in animals and bringing other side effects. In the meantime, the present invention provides an advantage of nature-friendly effect by using the composition containing natural bacteriophage as an active ingredient.

BRIEF DESCRIPTION OF THE DRAWINGS

The application of the preferred embodiments of the present invention is best understood with reference to the accompanying drawings, wherein:

FIG. 1 is an electron micrograph showing the bacteriophage STP-1.

FIG. 2 is a diagram illustrating the killing activity of the bacteriophage STP-1 against *Salmonella Typhimurium*.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Hereinafter, the present invention is described in detail.

The present invention provides a composition comprising the bacteriophage isolated from nature and characterized by having the ability to infect *Salmonella Typhimurium* so as to kill the same, and a method for preventing and treating *Salmonella Typhimurium* infection using the said composition.

The bacteriophage used as the active ingredient in the composition of the present invention is the bacteriophage STP-1 having DNA represented by SEQ. ID. NO: 1 as its genome. The bacteriophage STP-1 was isolated by the present inventors and deposited at Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology on Sep. 5, 2011 (Accession No: KCTC 12012BP).

The present invention also provides a disinfectant, a water additive, and a feed additive that can be used for the prevention or treatment of *Salmonella Typhimurium* infection.

The bacteriophage STP-1 included in the composition of the present invention is capable of killing *Salmonella Typhimurium* efficiently, suggesting that the bacteriophage is effective in prevention or treatment of salmonellosis caused by *Salmonella Typhimurium*. Therefore, the composition of the present invention is useful for the prevention and treatment of salmonellosis caused by *Salmonella Typhimurium*. The term "salmonellosis" in this invention generally indicates all the symptoms caused by *Salmonella* infection including fever, headache, diarrhea, vomiting, etc.

The term "treat" or "treatment" in this description indicates (i) to suppress salmonellosis caused by *Salmonella Typhimurium*; and (ii) to relieve salmonellosis caused by *Salmonella Typhimurium*.

In this invention, the term "isolation" or "isolated" indicates the separation of bacteriophage from nature by using diverse experimental techniques and the process confirming the characteristics of the bacteriophage that can distinguish the bacteriophage itself from others. This term further includes the course of proliferating the bacteriophage by using biotechnology in order to make it a useful form.

The bacteriophage of the present invention includes the bacteriophage STP-1 and its variants as well. In this invention, "variants" indicate those bacteriophages which have the same genotypic/genotypic characteristics as the bacteriophage STP-1 even though there is minor variation in genomic sequencer or polypeptide encoding genetic information. Needless to say, the variants herein include polymorphic variants, too. It is preferred for those variants to have the same or equivalent biological function to the bacteriophage STP-1.

The composition of the present invention can include pharmaceutically acceptable carriers such as lactose, dextrose, sucrose, sorbitol, mannitol, starch, acacia rubber, calcium phosphate, alginate, gelatin, calcium silicate, microcrystalline cellulose, polyvinyl pyrrolidone, cellulose, water, syrup, methyl cellulose, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate and mineral oil, but not always limited thereto. The composition of the present invention can additionally include lubricants, wetting agents, sweetening agents, flavors, emulsifiers, suspensions and preservatives.

The composition of the present invention contains the bacteriophage STP-1 or the variants thereof as an active ingredient. At this time, the bacteriophage STP-1 or the variants thereof are included at the concentration of 1×10^1 pfu/ml $\sim 1 \times 10^{30}$ pfu/ml or 1×10^1 pfu/g $\sim 1 \times 10^{30}$ pfu/g, and more preferably at the concentration of 1×10^4 pfu/ml $\sim 1 \times 10^{15}$ pfu/ml or 1×10^4 pfu/g $\sim 1 \times 10^{15}$ pfu/g.

The composition of the present invention can be formulated by the method that can be performed by those in the art by using a pharmaceutically acceptable carrier and/or excipient in the form of unit dose or in multi-dose containers. The formulation can be in the form of solution, suspension, or emulsion in oil or water-soluble medium, extract, powder, granule, tablet or capsule. At this time, a dispersing agent or a stabilizer can be additionally included.

The composition of the present invention can be produced in the form of a disinfectant, a water additive, and a feed additive, but not always limited thereto.

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples, Experimental Examples and Manufacturing Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Example 1

Isolation of Bacteriophage that can Destroy *Salmonella Typhimurium*

A bacteriophage capable of killing *Salmonella Typhimurium* was isolated from nature or from animal samples. *Salmonella Typhimurium* used for the isolation was *Salmonella Typhimurium* ST2, which was isolated previously by the present inventors and then identified as *Salmonella Typhimurium* by the inventors.

Collected samples were loaded in TSB (Tryptic Soy Broth) medium (casein digest, 17 g/L; soybean digest, 3 g/L; dex-

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trose, 2.5 g/L; NaCl, 5 g/L; dipotassium phosphate, 2.5 g/L) inoculated with *Salmonella Typhimurium* (1/1000), followed by shaking culture for 3~4 hours at 37° C. Upon completion of the culture, centrifugation was performed at 8,000 rpm for 20 minutes and the supernatant was recovered. *Salmonella Typhimurium* was inoculated in the recovered supernatant (1/1000), followed by shaking culture for 3~4 hours at 37° C. This procedure was repeated 5 times in total in order to increase bacteriophage titer if bacteriophage was included in the sample. After repeating the process 5 times, the culture solution proceeded to centrifugation at 8,000 rpm for 20 minutes. Then, the supernatant was filtered using 0.45 µm filter. The obtained filtrate was investigated by using general spot overlay assay to see whether bacteriophage that could kill *Salmonella Typhimurium* was included.

Spot overlay assay was performed as follows. *Salmonella Typhimurium* was inoculated in TSB medium (1/1000), followed by shaking culture at 37° C. overnight. Then, 3 ml of the obtained *Salmonella Typhimurium* culture solution (OD₆₀₀: 2.0) was spread on TSA (Tryptic Soy Agar) plate medium (casein digest, 15 g/L; soybean digest, 5 g/L; NaCl, 5 g/L; agar, 15 g/L). The plate medium stayed on clean bench for about 30 minutes to let the spread solution is dried. After drying, 10 µl of the prepared filtrate was spotted on the plate whereon *Salmonella Typhimurium* was spread, which was dried as it was for 30 minutes. After drying, the plate was incubated at 37° C. for a day. It was then examined whether the clear zone was formed on the spot where the filtrate was spotted. If the clear zone was formed thereon, it suggested that the bacteriophage that could kill *Salmonella Typhimurium* was included therein. According to this procedure, the filtrate containing the bacteriophage that could destroy *Salmonella Typhimurium* could be obtained.

Pure bacteriophage was isolated from the filtrate confirmed to contain the bacteriophage capable of killing *Salmonella Typhimurium*. The isolation of pure bacteriophage was performed by plaque assay. More precisely, one of plaques formed from plaque assay was recovered by using a sterilized tip, which was then added to *Salmonella Typhimurium* culture solution, followed by culture for 4~5 hours. Upon completion of the culture, centrifugation was performed at 8,000 rpm for 20 minutes to obtain supernatant. *Salmonella Typhimurium* culture solution was added to the obtained supernatant at the ratio of 1:50, followed by further culture for 4~5 hours. To increase the number of bacteriophage, this procedure was repeated at least 5 times and then centrifugation was performed at 8,000 for 20 minutes to obtain supernatant. Plaque assay was performed with the supernatant. Generally, the pure bacteriophage isolation cannot be accomplished simply by performing the above procedure once. Thus, the previous steps were repeated again using one of plaques formed from plaque assay. After repeating the procedure at least 5 times, the solution comprising pure bacteriophage was obtained. The repetition of this pure bacteriophage isolation processes was not finished until the sizes and shapes of plaques were all similar. The pure bacteriophage isolation was confirmed at last by the observation under electron microscope. If pure bacteriophage was not confirmed, the above processes were repeated again. Observation under electron microscope was performed by the conventional method, which was as follows: copper grid was soaked in the solution containing pure bacteriophage, followed by negative staining with 2% uranyl acetate and drying thereof; and morphology was observed by taking pictures with transmission electron microscope.

The solution containing pure bacteriophage was purified as follows. *Salmonella Typhimurium* culture solution was added to the pure bacteriophage solution at the ratio of 1:50, fol-

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lowed by culture for 4~5 hours. Upon completion of the culture, centrifugation was performed at 8,000 rpm for 20 minutes to obtain supernatant. To obtain enough amount of bacteriophage, the said process was repeated 5 times in total. The final supernatant was filtered with 0.45 µm filter, followed by precipitation by using polyethylene glycol (PEG). Particularly, PEG and NaCl were added to 100 ml of the filtrate (10% PEG 8000/0.5 M NaCl), which stood at room temperature for 2~3 hours. Then, centrifugation was performed at 8,000 rpm for 30 minutes to obtain bacteriophage precipitate. The obtained bacteriophage precipitate was suspended in 5 ml of buffer (10 mM Tris-HCl, 10 mM MgSO₄, 0.1% Gelatin, pH 8.0). This solution was called bacteriophage suspension or bacteriophage solution.

At last, purified pure bacteriophage was obtained and this bacteriophage was named bacteriophage STP-1, which was then deposited at Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology on Sep. 5, 2011 (Accession No: KCTC 12012BP). The electron micrograph of bacteriophage STP-1 is presented in FIG. 1.

Example 2

Extraction of Bacteriophage STP-1 Genome and Sequencing Thereof

Bacteriophage STP-1 genome was extracted as follows using the bacteriophage suspension obtained in Example 1. To eliminate *Salmonella Typhimurium* DNA and RNA which might be included in the suspension, DNase I and RNase A were added to 10 and of the bacteriophage suspension (200 U each), which stood at 37° C. for 30 minutes. 30 minutes later, to neutralize DNase I and RNase A activity, 500 µl of 0.5 M ethylenediaminetetraacetic acid (EDTA) was added, which stood for 10 minutes. The solution stood at 65° C. for another 10 minutes, then 100 µl of proteinase K (20 mg/ml) was added, followed by incubation at 37° C. for 20 minutes to break the outer wall of the bacteriophage. Then, 500 µl of 10% sodium dodecyl sulfate (SDS) solution was added thereto, followed by incubation at 65° C. for one hour. One hour later, 10 ml and of the mixed solution comprising phenol chloroform:isoamylalcohol at the volumetric ratio of 25:24:1 was added thereto and the solution was well mixed. Centrifugation was performed at 13,000 rpm for 15 minutes to separate layers, among which the upper most layer was obtained. Isopropyl alcohol was added to the obtained layer at the volume ratio of 1.5, followed by centrifugation at 13,000 rpm for 10 minutes to precipitate genome. The precipitate was recovered, to which 70% ethanol was added, followed by centrifugation at 13,000 rpm for 10 minutes. The washed precipitate was collected and vacuum-dried, which was then dissolved in 100 µl of water. Genome of the bacteriophage STP-1 was obtained by repeating the above processes.

The genomic sequence of the bacteriophage STP-1 was analyzed with the obtained genome at National Instrumentation Center for Environmental Management, Seoul National University, by using shotgun library construction. Particularly, the bacteriophage genome was cut by random shearing technique using Nebulizer to obtain DNA fragments (1~6 kbp), which proceeded to end-repairing. The repaired DNA proceeded to electrophoresis on agarose gel. Then, gDNA fragments (inserts) in 3~5 kbp were obtained. The obtained DNA fragments of the bacteriophage genome were inserted in pC31 vector by using T4 ligase (ligation) to establish library. The recombinant plasmid introduced with the DNA fragment of the bacteriophage genome was inserted in DH10B', a kind of *E. coli*, via transfection. The transformant

harboring the plasmid was cultured, from which the plasmid containing the gene fragment was extracted by using plasmid purification kit (iNtRON). The size of the DNA fragment included in the plasmid was confirmed by electrophoresis and the final effective clones were selected. The plasmid of the selected clone was recovered, followed by gene sequencing. Contig map was made using the obtained gene sequences by the conventional method. The total gene sequence in 157,662 bp was analyzed by using primer walking. The confirmed genomic sequence of the bacteriophage STP-1 was presented by SEQ. ID. NO: 1.

Based on the genomic sequence of the bacteriophage STP-1, similarity to those sequences of the reported bacteriophages was investigated by using BLAST. As a result, the genomic sequence of the bacteriophage STP-1 demonstrated the highest homology (93%) with the sequence of *E. coli* bacteriophage vB_EcoM_CBA120 (*Escherichia phage* vB_EcoM_CBA120) (GenBank Accession No. JN593240.1). The total genomic sequence of the *E. coli* bacteriophage vB_EcoM_CBA120 was 157,340 bp, which was similar to the size of the bacteriophage STP-1. Open reading frame (ORF) of the total gene sequence of the bacteriophage STP-1 was analyzed by using ORF finder. As a result, the bacteriophage STP-1 had total 207 ORFs, which was different from the *E. coli* bacteriophage vB_EcoM_CBA120 (203 ORFs). Among ORFs of the bacteriophage STP-1, multiple numbers of them were confirmed not to exist in the sequence of the *E. coli* bacteriophage vB-EcoM_CBA120.

Therefore, it can be concluded that the bacteriophage STP-1 is a novel bacteriophage which is completely different from any of the reported bacteriophages.

Example 3

Killing Activity of Bacteriophage STP-1 to *Salmonella Typhimurium*

Killing activity of the isolated bacteriophage STP-1 to *Salmonella Typhimurium* was investigated. For the investigation, clear zone formation was first observed by spot overlay assay according to the same manner as described in Example 1. 50 strains of *Salmonella Typhimurium* were used in this investigation. They were isolated and identified by the present inventors as *Salmonella Typhimurium* earlier. The bacteriophage STP-1 was confirmed to have the ability to kill all the *Salmonella Typhimurium* used in this experiment. The result of this investigation is presented in FIG. 2. In addition, killing activity of the bacteriophage STP-1 to *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus agalactiae*, *Streptococcus mitis*, *Streptococcus uberis*, *Escherichia coli*, and *Pseudomonas aeruginosa* was further investigated. As a result, it was confirmed that the bacteriophage STP-1 did not have killing activity against those bacteria.

From the above results, it was confirmed that the bacteriophage STP-1 can be used as an active ingredient of the composition formulated for the purpose of prevention and treatment of *Salmonella Typhimurium* infection.

Example 4

Application Example of Bacteriophage STP-1 for Preventing *Salmonella Typhimurium* Infection

100 μ l of the bacteriophage STP-1 solution (1×10^8 pfu/ml) was loaded to a tube containing 9 ml of TSB medium. Another tube containing 9 ml of TSB medium alone was also prepared.

The *Salmonella Typhimurium* culture solution was added to each tube (OD_{600} : 0.5). After *Salmonella Typhimurium* bacteria were added to those tubes, they were all transferred to 37° C. incubator, followed by shaking culture, during which the growth of *Salmonella Typhimurium* was observed. As shown in Table 1, the growth of *Salmonella Typhimurium* was suppressed in the tube treated with the bacteriophage STP-1 solution. In the meantime, the growth of *Salmonella Typhimurium* was not inhibited in the bacteriophage free tube.

TABLE 1

| Suppression of <i>Salmonella Typhimurium</i> growth | | | |
|---|----------------|-----------------|-----------------|
| | OD_{600} | | |
| | Culture 0 min. | Culture 15 min. | Culture 60 min. |
| Without bacteriophage solution | 0.5 | 0.7 | 1.5 |
| With bacteriophage solution | 0.5 | 0.1 | 0.05 |

The above results indicate that the bacteriophage STP-1 of the present invention not only suppresses the growth of *Salmonella Typhimurium* bacteria but also even destroys them, so that it can be used as an active ingredient for the composition formulated for the purpose of prevention of *Salmonella Typhimurium* infection.

Example 5

Treatment Example of *Salmonella Typhimurium* Infectious Disease Using Bacteriophage STP-1

Treating effect of the bacteriophage STP-1 was investigated in pigs infected with *Salmonella Typhimurium*. Particularly, two pig groups were arranged and each group had 5 weaning pigs at 25 days of age. The test animals were raised separately in laboratory animal facilities (1.1 m \times 1.0 m), during which experiment was performed for 14 days. Environment was controlled in the thermal insulation facility. Temperature and humidity were regularly controlled and the floor of the pig room was cleaned every day. On the 7th day from the experiment started, all the pigs were orally administered with *Salmonella Typhimurium* solution. The *Salmonella Typhimurium* solution for oral administration was prepared as follows: *Salmonella Typhimurium* was cultured in TSB medium at 37° C. for 18 hours; The cells were recovered; and the recovered cells were suspended in saline (pH 7.2) at the concentration of 10^{11} CFU/ml. A day after the administration of *Salmonella Typhimurium*, the pigs were orally administration with the bacteriophage STP-1 (10^9 PFU) (bacteriophage solution treated group), twice a day, by the same method as used for the oral administration of *Salmonella Typhimurium* solution. The control group pigs were not treated with the bacteriophage STP-1 (bacteriophage-non-treated group). Feeds and drinking water were equally given to both the control and the experimental groups. All the test animals had been observed every day since they were administered with *Salmonella Typhimurium* to see if they had diarrhea or not. The condition of diarrhea was examined by using diarrhea index. Diarrhea index was made by Fecal Consistency (FC) score (normal: 0,

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loose feces: 1, moderate diarrhea: 2, and explosive diarrhea: 3). Diarrhea index was continuously increased in the control group pigs for 4 days from the administration of *Salmonella Typhimurium*, but the index was slowly decreased afterward. In the meantime, diarrhea index was constantly reduced in the experimental group pigs from the next day after the administration of *Salmonella Typhimurium* (Table 2).

TABLE 2

| | Diarrhea index | | | | | | |
|---|---|-----|-----|-----|-----|-----|-----|
| | Days after <i>Salmonella Typhimurium</i> administration | | | | | | |
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control group (bacteriophage non-treated) | 0.4 | 0.8 | 1 | 0.6 | 0.6 | 0.4 | 0.3 |
| Experimental group (bacteriophage treated) | 0.4 | 0.3 | 0.3 | 0.2 | 0.2 | 0 | 0 |

The above results indicate that the bacteriophage STP-1 of the present invention is very effective in the treatment of infectious disease caused by *Salmonella Typhimurium*.

Example 7

Preparation of Feed Additive and Feed

Feed additive containing bacteriophage STP-1 at the concentration of 1×10^9 pfu/g was prepared with bacteriophage STP-1 solution. The preparation method was as follows. Bacteriophage STP-1 solution was evenly sprayed on maltodextran at the proper weight ratio, which was then vacuum-dried at room temperature, followed by pulverization into fine powders. Silica was added thereto at the weight ratio of 5% and the mixture was well mixed. For the drying process, either reduced pressure drying, drying at elevated temperature, or freeze drying could be used. For the control, bacteriophage free feed additive was also prepared by spraying buffer (10 mM Tris-HCl, 10 mM $MgSO_4$, 0.1% Gelatin, pH 8.0) alone which was the same buffer that was used for the preparation of bacteriophage solution, instead of bacteriophage solution.

The above two feed additives were mixed with feed for hog respectively at the weight ratio of 1:1,000.

Example 8

Preparation of Water Additive and Disinfectant

A water additive and a disinfectant were prepared by the same method because both were formulated in the same form and have only difference in their use. The water additive (or disinfectant) containing bacteriophage STP-1 at the concentration of 1×10^9 pfu/ml was prepared. The method of preparation of water additive (or disinfectant) was as follows. Bacteriophage STP-1 was added to the buffer which was generally used for the preparation of bacteriophage solution at the concentration of 1×10^9 pfu/ml and then well mixed. For the control, the buffer itself was used as the bacteriophage free water additive (or disinfectant).

The prepared two different water additives (or disinfectants) were diluted with water at the ratio of 1:1,000, resulting in the final water additive or disinfectant.

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Example 9

Investigation of Feeding Efficacy in Pig Farming

Improvement of feeding efficacy in pig farming was investigated by using the feeds, water, and disinfectants prepared in Examples 7 & 8. In particular, this investigation was performed by observing death rate. 30 piglets were divided into three groups (10 piglets/group) (group A: supplied with the bacteriophage containing or free feeds; group B: supplied with the bacteriophage containing or free water; group C: treated with the bacteriophage containing or free disinfectants). The investigation was performed for 4 weeks. Each group was divided into two subgroups of 5 piglets. Those subgroups were either treated with bacteriophage STP-1 (subgroup ①) or not treated with bacteriophage STP-1 (subgroup ②). The test piglets were 20 days old. Each group piglets were raised in an isolated cage separated from each other at regular intervals. Each subgroup was sorted and marked as shown in Table 3.

TABLE 3

| | Subgroup sorting and marking in feeding efficacy test on pig farming | |
|--------------|--|----------------------|
| | Subgroup sorting and marking | |
| | Bacteriophage STP-1+ | Bacteriophage STP-1- |
| Feed | A-① | A-② |
| Water | B-① | B-② |
| Disinfectant | C-① | C-② |

The piglets were supplied with the feeds prepared in Example 7 and the water prepared in Example 8 according to the conventional method as shown in Table 3. Disinfection was performed with the conventional disinfectant and the disinfectant of the present invention by taking turns, three times a week. The day when the disinfectant of the present invention was sprayed on, the conventional disinfectant was not used. The results are shown in Table 4.

TABLE 4

| Group | Death rate (%) |
|-------|----------------|
| A-① | 0 |
| A-② | 20 |
| B-① | 0 |
| B-② | 40 |
| C-① | 0 |
| C-② | 40 |

From the above results, it was confirmed that the feeds, water and disinfectants prepared according to the present invention could help to reduce death rate in pig farming. Therefore, it was concluded that the composition of the present invention was effective in the improvement of feeding efficacy in pig farming.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.

SEQUENCE LISTING

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<211> LENGTH: 157662

<212> TYPE: DNA

<213> ORGANISM: Bacteriophage STP-1

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What is claimed is:

1. A method for treating *Salmonella Typhimurium* infection in a subject animal in need thereof, which comprises the step of administering a composition comprising an isolated bacteriophage STP-1 having a genome comprising SEQ. ID. NO: 1 and deposited under Accession No: KCTC 12012BP as an active ingredient to a subject animal.

2. The method for treating *Salmonella Typhimurium* infection according to claim 1, wherein the composition is administered to a subject animal in the form of a feed additive, a water additive, or a disinfectant.

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